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A COMPARISON OF FREEZE-DRIED BEEF MUSCLES OF HIGH OR LOW ULTIMATE pH

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Some of the deleterious effects of freeze-drying on the texture of beef have been obviated by using muscles with a high ultimate pH. Pre-slaughter injection of adrenaline raised the ultimate pH to 6·7. After freeze-drying the treated beef was more tender, more juicy and less woody than controls at pH 5·6. A resultant increase in water-holding capacity and rehydratability was demonstrated by myofibrillar swelling and increased fibre diameter. Dehydrated beef with a high ultimate pH showed less deterioration during storage at 37° in the attributes texture, flavour and colour.

Introduction

Considerable technological advances in the freeze-drying of foodstuffs have been made in recent years. As a result, the process of Accelerated Freeze-Drying (AFD)[1] has become commercially possible. Nevertheless the process causes changes in some products which adversely affect their quality. Raw meat subjected to the AFD process is usually tougher and drier than the original meat and in addition has a characteristic 'woody' texture. These undesirable features are paralleled by a reduction in the water-holding capacity of the proteins after drying.[2]

The protein/water-holding relationship of muscle is affected to a considerable degree by the extent of pH fall during *post mortem* glycolysis. Thus a high ultimate pH is associated with an increase in water-holding capacity.[3, 4] An ultimate pH greater than 6·5 can be induced experimentally in musculature by pre-slaughter injection of animals with adrenaline.[5] Preliminary work[6] on rabbit had shown that after being freeze-dried, muscle with an ultimate pH of 6·7 had a higher water-holding capacity, was more tender and less woody than muscle with a normal pH of 5·9. These experiments have now been extended to beef. In addition to examining the effect of high ultimate pH on the texture of the beef after freeze-drying the effect on subsequent storage has also been studied.

Experimental

Injection of steers and sample preparation

Identical twin steers (of Aberdeen Angus–Jersey breed) were employed in the hope of lessening differences due to inter-animal variation. One of these was injected subcutaneously

with adrenaline (100 μg./kg.) at 24 h. and again 4 h. pre-slaughter; the other twin, which was not injected, served as a control. The left hand side of each carcass was dissected into its constituent anatomical muscles. *Semimembranosus, biceps femoris, psoas major, longissimus dorsi* (lumbar), *longissimus dorsi* (thoracic) and deep pectoral muscles were held at +1° for 7 days before being frozen at −20°. The values for the ultimate pH of the fresh muscle are compared in Table I, from which the effect of adrenaline in depleting the glycogen reserves, and thereby in raising the ultimate pH, will be apparent. The pH increment in the *psoas major* was slightly greater, and that in deep pectoral slightly less, than those obtained with the other four muscles.

To prepare the meat for dehydration, slices 12 mm. thick were cut from the whole frozen muscle. Each set of six slices taken consecutively along the muscle comprised one unit. From each unit, two slices wrapped in polythene pouches were held frozen at −20° and the remainder were freeze-dried by the AFD process. The adrenaline treatment appeared to have little effect on the length of the drying run since the average time for the control was 5½ h. and the treated 5¾ h.

After being dried each unit was separately sealed in a can and gas packed under nitrogen. Samples were held at room temperature, at 0 and 37°.

For convenience the frozen beef from the adrenaline injected steer will be referred to as 'treated' and the beef from the other twin as 'control'.

Cooking and tasting

Dehydrated whole steaks were reconstituted by immersion in water for 20 min. During this time corresponding frozen steaks were thawed out in water. The samples were then casseroled (in the immersion water) in an oven at 185° for 1½ h. To assess texture, a taste panel (8 members) was asked to place the samples in order of increasing toughness and dryness, and also to indicate 'woodiness' when it was detected. Each taster received treated and control samples, both frozen and dehydrated, but the order in which they were tasted was random. Only one muscle was assessed at each sitting. For estimation of stored samples, an arbitrary scale was used (0 very tough; 6 tender).

Tenderometer measurements

Rehydrated and thawed steaks, wrapped tightly in aluminium foil, were cooked by steaming for 1 h. After cooling, pieces 1 cm. wide and 0·5 cm. thick were cut; the length of the piece depended on the original thickness of the steak. The pieces were cut so that the direction of the fibres was always at right angles to the shearing edge of the tenderometer. The instrument used was that described by Grünewald.[7] The toughness of a sample was determined by the work done by the tenderometer (under a constant load of 15 kg.) in shearing the pieces of meat. (Results were calculated for pieces of meat 1 cm. thick.)

Reconstitution

The influence of ultimate pH on the reconstitution of the dehydrated beef was examined by the following procedure. The dehydrated samples were first ground by passing them through a mincer with a plate having 4-mm. holes, and then washed several times with cold diethyl ether to remove all the fat. The ether was evaporated by suction in a Buchner funnel and the dried powder passed through a No. 40 mesh sieve, which removed large pieces of connective tissue and fibre clumps (approximately one-third of the whole dried powder). Of the powder

Table I

Ultimate pH of fresh muscles from adrenaline-treated and control identical twin steers

Muscle	Treated	Control
Semimembranosus	6·80	5·68
Biceps femoris	6·70	5·50
Psoas major	7·01	5·52
Longissimus dorsi (lumbar)	6·75	5·60
,, ,, (thoracic)	6·80	5·60
Deep pectoral	6·52	5·60

which passed through the sieve, 2 g. were weighed accurately in a centrifuge tube and mixed with 20 ml. of water. After being kept for 20 min. the tube was centrifuged at 2500 *g* for 5 min. The supernatant was discarded and the residue drained for 10 min. and reweighed. The reconstitution ratio was determined as the weight of water absorbed per g. of dry meat powder.

Preparation of myofibrils and measurement of water binding

Myofibrils were prepared by homogenising 30 g. of meat in ice-cold 0·1M-potassium chloride in a high-speed blendor for 2 min. From control dehydrated material, however, low yields were obtained and further periods of homogenising were necessary to prepare myofibrils in sufficient quantities for determining pH/water-binding relationships. The myofibrils were washed with 0·1M-potassium chloride until free of soluble sarcoplasmic proteins. A suspension with a concentration of 5–6 mg. fibrillar protein/ml. was prepared in 0·1M-potassium chloride. Measurement of the effect of pH on the water binding of the myofibrils was carried out on 10-ml. portions by adjusting the pH of the suspension with 0·1N-hydrochloric acid or -sodium hydroxide. After being kept for 15 min. to allow the myofibrils to equilibrate to the required pH, the suspensions were centrifuged at 1200 *g* for 5 min. The myofibril layer was weighed after decanting and draining the supernatant. Protein concentration was usually determined on a solution of myofibrils in 0·01N-hydrochloric acid either by measuring the absorption at 220 mμ or by Lowry's modification[8] of the method of Folin. In both cases standardisation was first carried out by the usual Kjeldahl procedure.

Histological studies

A series of slices from each muscle were selected for histology. Samples were obtained after being frozen at −20° and after dehydration. The samples were removed with a No. 8 cork borer and, in order to provide valid comparisons, were obtained from the same relative position in each slice.

Measurements of fibre diameter were carried out on teased preparations of the frozen samples. The cylinders of frozen tissue removed with a cork borer were allowed to thaw at room temperature before a portion was removed for teasing. The method described by Hiner and his co-workers[9] was employed. By means of an eyepiece micrometer scale three readings for each of twelve fibre fragments were obtained. The readings were taken at points approximately 100 μ apart. The ends of fragments and those showing excessive damage due to teasing were avoided. (Measurements of fibre diameter of unfrozen muscle were not materially different from those obtained with frozen tissue.)

Similar measurements were also made on reconstituted tissue. Samples were removed from the dehydrated slices in the same way as from the frozen material. The tissue cylinders were cut into slices approximately 2–3 mm. in thickness and reconstituted in distilled water. Reconstitution was carried out at room temperature in a vacuum of 600 mm. Hg. After 30 min. the samples were removed from the vacuum chamber and placed on filter paper for 5 min. to remove excess water. Fibre diameters were determined on teased preparations as above.

Moisture content

This was determined by drying minced dehydrated samples in a vacuum oven at 70° and less than 3 mm. pressure for 5 h.

Glucose estimation

Glucose was extracted by blending 0·5 g. of dehydrated beef with 3 ml. of 2N-perchloric acid and 3 ml. of water. After removal of the precipitated protein by centrifuging, 2 ml. of the supernatant were neutralised to pH 6 with 2N-potassium hydroxide and made up to 15 ml. The solution was then chilled to precipitate the potassium perchlorate which was removed by filtration and the filtrate was further diluted to 3 times its volume. Glucose was determined on 1-ml. portions by a modification[10] of the glucose oxidase method of Saifer & Gerstenfeld[11] which is specific for glucose.

Degree of browning

Washed homogenates were treated with acetone to remove the water, and the acetone was evaporated by suction at a vacuum pump. The reflectance of the acetone-dried powder was measured at 400 mμ on a Unicam SP 500 with a reflectance attachment.

Results

Tasting

From the results given in Table II the effectiveness of high ultimate pH in improving the texture of beef is clearly demonstrated. The treated (frozen) sample was placed first ($p = 0.05$) in tenderness and juiciness. This improvement was maintained after drying, the treated dehydrated sample being placed second ($p = 0.05$). The control (frozen) sample, which was third, was only slightly different from the control dehydrated meat except in the incidence of 'woodiness' which was greatest in the latter.

Table II

Taste panel results

The sum of the rankings given by eight tasters for treated and control (both frozen and dehydrated) for tenderness and juiciness

Muscle	Treated		Treated dehydrated		Control		Control dehydrated	
	Tenderness	Juiciness	Tenderness	Juiciness	Tenderness	Juiciness	Tenderness	Juiciness
Semimembranosus	12	13	16	19	23	24	24	23
Biceps femoris	11	15	15	22	26	20	25	22
Psoas major	12	12	14	16	23	25	25	25
Longissimus dorsi (lumbar)	19	11	17	14	19	26	21	24
,, ,, (thoracic)	11	10	12	20	26	22	25	25
Deep pectoral	10	10	16	19	25	25	27	24
Total	75	71	90	110	142	142	147	143
No. of times 'woodiness' was indicated	2		9		10		16	

Tenderometer measurements

Objective measurement of toughness by tenderometer confirmed the findings of the taste panel (Table III). As before, the treated frozen meat was the most tender. The treated dehydrated muscles were in most cases more tender than the corresponding control (frozen) muscles; and, in all cases, more tender than corresponding control dehydrated muscles. The results also show a slight, but consistent, tendency to greater toughness in the control after dehydration. It is interesting to note that the effect of the treatment varied for the different muscles. The increase in tenderness of the *biceps femoris* was slight, whereas the *semimembranosus* (which was the toughest muscle in the control animal) was very tender in the treated animal and comparable to the *psoas*.

Reconstitution

An increase in the amount of water absorbed during reconstitution was found in all the treated dehydrated muscles in comparison with corresponding control dehydrated material. The method used, because of the removal of fat and the uniform particle size, gave consistent

Table III

Measurement of work done (ergs × 10^6/cm.) by a tenderometer in shearing meat

Muscle	Treated	Treated dehydrated	Control	Control dehydrated
Semimembranosus	9·8	13·4	24·5	25·6
Biceps femoris	12·1	13·6	12·6	15·2
Psoas major	9·2	9·5	12·9	12·4
Longissimus dorsi (lumbar)	11·5	11·8	15·2	17·7
,, ,, (thoracic)	6·9	9·1	12·9	17·2
Deep pectoral	14·8	18·3	18·6	19·4

Table IV

Reconstitution ratios (g. H_2O/g. dry wt.) of dehydrated muscle from adrenaline-treated and control steers measured after 20 min. reconstitution and centrifuging at 2500 g for 5 min.

Muscle	At ultimate pH of muscle Treated dehydrated	At ultimate pH of muscle Control dehydrated
Semimembranosus	3·10	1·99
Biceps femoris	3·12	1·96
Psoas major	3·58	2·08
Longissimus dorsi (lumbar)	3·22	1·98
,, ,, (thoracic)	3·34	2·02
Deep pectoral	3·65	1·95

results for the various muscles from the control animal as shown in Table IV. The fact that the muscles from the treated animal absorbed markedly different amounts of water can be taken as an indication that the muscles responded differently to the adrenaline treatment.

Myofibrillar swelling

The results in Fig. 1 show the pH/swelling curves, averaged for all muscles, and the curves for individual muscles in Fig. 2. It can be seen from both these figures that the myofibrils from the treated muscle absorbed more water than those from the control, this being true throughout the range of environmental pH studied. It was found, however, by microscopical examination with phase contrast illumination at a magnification ×675, that, although the myofibrils prepared from the control muscles remained separated throughout the pH range used, the treated myofibrils clumped together when the pH was lowered to less than 6·5 by the addition of 0·1N-hydrochloric acid. This altered their sedimentation behaviour during centrifuging. Accordingly, comparison between the myofibrils of the treated and control muscles could be made only when they were in a similar separated state. The effect of dehydration on the water holding of the myofibrils is also shown in the curves. The myofibrils from both treated and control dehydrated material absorbed less water than their non-dehydrated counterparts. But the treated dehydrated absorbed more water than the control dehydrated at pH 6·5 and above thus indicating the improved water-holding capacity of dehydrated meat with a high ultimate pH.

The results in Fig. 2 illustrate that the different muscles responded in varying degrees to a high ultimate pH. The pH/swelling curve for the *longissimus dorsi* (lumbar) was practically the same for the treated (frozen), treated (dehydrated) and control (frozen) samples. However, all the other muscles from the treated meat absorbed more water at pH 6·5 than corresponding controls; the greatest increase was found with the *psoas* and the *biceps femoris*. With the exception of *longissimus dorsi* (lumbar) all the treated muscles have a lower water-holding capacity after dehydration, the drop being approximately the same for each muscle. Similarly the water-holding capacity of all the control muscles was lower after dehydration. (In this particular figure the curves for three muscles only have been shown in order to avoid confusion: the other three muscles give a similar picture.)

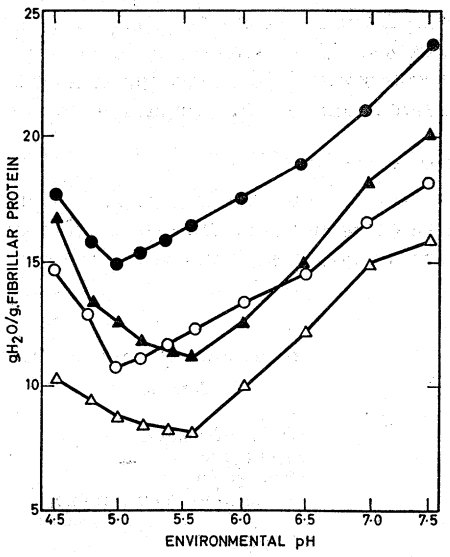

FIG. 1.—*Effect of environmental pH on the water-holding capacity of myofibrils prepared from muscles with high or normal ultimate pH*

(Average results of all muscles)

—●— Treated: ultimate pH 6·7
—○— Treated, dehydrated: ultimate pH 6·7
—▲— Control: ultimate pH 5·6
—△— Control, dehydrated: ultimate pH 5·6

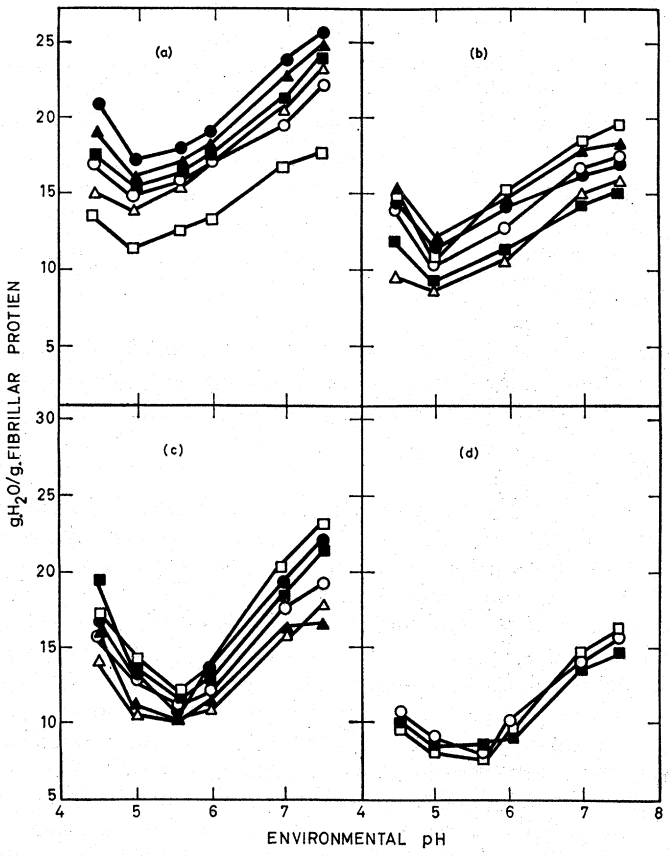

FIG. 2.—*Effect of environmental pH on water-holding capacity of myofibrils prepared from individual muscles with high or normal ultimate pH*

(a) Treated frozen, (b) treated dehydrated, (c) control frozen, (d) control dehydrated

—●— *Biceps femoris*
—▲— *Psoas major*
—■— *Semimembranosus*
—○— *Longissimus cervicus*
—△— Deep pectoral muscle
—□— *Longissimus dorsi*

In addition to the increase in the water-holding capacity of the rehydrated muscles of the treated animal, an increase in the ease of breakdown of fibres to fibrils was observed. To estimate this difference, a weighed quantity of the fat-free powder used in reconstitution ratio measurements was homogenised with 0·1M-potassium chloride in a small blendor for 5 min. The resulting homogenate was centrifuged and the myofibril layer, which was removed as accurately as possible, was washed three times in 0·1M-potassium chloride by centrifuging and resuspension. Protein determinations were carried out on the myofibrils and the amount of fibrillar protein prepared from 1 g. of dried meat is shown in Table V. Clearly the control rehydrated meat resisted breakdown to myofibrils to a greater extent than the rehydrated meat from the treated animal.

The effect of the pH of the homogenising medium on the swelling of the myofibrils has also been studied. Control and treated frozen meat was homogenised with 0·1M-potassium chloride in 0·03M-sodium glycerophosphate buffer at pH 5·6 or 6·7. After homogenisation, sodium hydroxide or hydrochloric acid was added to correct the pH to 5·6 or 6·7 and the homogenates were left overnight to equilibrate. The myofibrils were then washed and centrifuged in the appropriate buffer. The pH/swelling curves were obtained by the method previously described. The results in Fig. 3 show that, when meat of normal ultimate pH 5·6 was homogenised at pH 6·7, no increase in swelling at pH 7 was found, but when meat with high ultimate pH was homogenised at pH 5·6, there was a considerable reduction in water holding.

Table V

Yield of fibrillar protein (mg.) from 1 g. of fat-free dehydrated meat

	Control	Treated
Longissimus dorsi	152	210
Biceps femoris	127	224
Semimembranosus	149	234

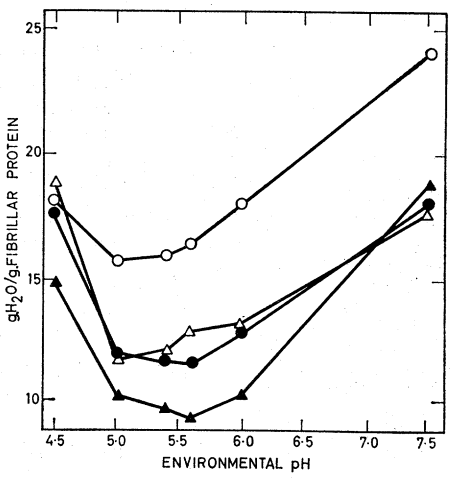

FIG. 3.—*Effect of adjusting the pH of homogenates of* biceps femoris *of high or normal ultimate pH, on the water-holding capacity of the myofibrils*

—○— Treated at pH 6·7
—●— Treated, adjusted pH 5·6
—△— Control, adjusted to pH 6·7
—▲— Control at pH 5·6

Histological studies

The mean fibre diameters of the muscles of the control and treated animals from the frozen and rehydrated states are shown in Table VI. The standard errors indicate a greater degree of scatter than was obtained with rabbit *longissimus dorsi*.

In studying rabbit *longissimus dorsi*[6] it had appeared that the mean fibre diameter increased as the ultimate pH of the muscle increased. In the work on beef muscles the greater variability between fibres may account partially for failure to observe a similar relationship. Nevertheless the induction of a high ultimate pH clearly increases rehydratability of muscle dried by the AFD process ; and this is reflected in the relatively greater fibre diameters of rehydrated muscle of high pH in comparison with corresponding muscle for the control steer.

Storage

The samples stored at −20° and 37° were examined after 4 months : the results for the *longissimus dorsi, biceps femoris* and *semimembranosus* are shown in Table VII.

Organoleptic assessment indicated that tenderness, juiciness and flavour of the meat with high ultimate pH were little affected by storage at 37° in comparison with corresponding material at −20°. The control samples of low ultimate pH, on the other hand, were considerably tougher and drier when stored at 37° than the corresponding material at −20° ; and the meat of low ultimate pH, whether held at −20° or 37°, was markedly less tender and juicy than the meat of high ultimate pH at either temperature. Measurement by tenderometer confirmed these findings on toughness, although the treated *semimembranosus* stored at 37° was found considerably tougher than the taste panel suggested.

It had been found previously that the dehydrated meat could readily be broken down to myofibrils by homogenising at high speed in 0·1M-potassium chloride. However, in these stored samples, even those held at −20°, the yield of myofibrils was very low. Accordingly the water-holding capacity was determined on fibres. A reduction in the water held by the control stored at 37° was found, but there was little diminution in the meat of high ultimate pH.

Table VI

Mean fibre diameter (μ) of muscle with normal and high ultimate pH following freezing and rehydration

Muscle	Frozen				Rehydrated				% Rehydration	
	Control	(SE)	Treated	(SE)	Control	(SE)	Treated	(SE)	Control	Treated
Semimembranosus	98·6	(23·4)	94·5	(17·5)	90·7	(27·2)	96·9	(15·1)	91·5	102·5
Biceps femoris	90·3	(17·2)	81·8	(12·5)	75·7	(14·3)	119·3	(13·5)	83·5	143·8
Longissimus dorsi	82·2	(18·2)	83·5	(22·0)	82·6	(17·0)	129·9	(15·3)	100·5	152·5
Psoas major	53·2	(11·9)	55·5	(12·0)	47·2	(11·2)	51·2	(6·8)	88·7	92·0

SE = standard error

Table VII

Effect of storage on dehydrated meat with ultimate pH 6·7 and 5·6

Muscle	Ultimate pH 6·7						Ultimate pH 5·6					
	Biceps femoris		*Semimembranosus*		*Longissimus dorsi*		*Biceps femoris*		*Semimembranosus*		*Longissimus dorsi*	
Storage temp., °C	−20	+37	−20	+37	−20	+37	−20	+37	−20	+37	−20	+37
Moisture, %	2·0	—	2·3	—	1·8	—	2·6	—	1·8	—	1·6	—
Glucose, mg./g.	0·06	0	0·10	0	0·07	0	2·9	0	3·4	0	2·6	0
Tenderness	—	4·5	3·6	3·1	4·3	4·1	3·9	2·8	—	2·0	3·4	2·0
Juiciness	—	3·2	3·9	3·4	4·4	4·4	2·8	2·8	—	3·9	4·3	3·1
Flavour	—	3·9	4·6	4·7	4·7	4·3	4·9	4·0	—	4·4	4·0	4·4
Tenderometer, ergs × 10^6	—	8·4	12·6	23·3	11·5	11·5	11·5	15·7	—	27·2	12·7	18·4
Reflectance at 400 mμ	—	0·375	0·240	0·390	0·127	0·351	0·249	0·435	—	0·565	0·152	0·497
Water holding at pH 6·5, g. H_2O/g. protein	—	6·7	8·9	9·0	10·8	10·3	8·3	6·2	—	6·6	9·8	8·2

Although the glucose content of the treated sample was low compared with that of the control, both browned on storage. The degree of browning was greater in the latter case and the general appearance was inferior.

Discussion

The main intention of the experiment with beef was to assess whether or not the beneficial effects of a high ultimate pH in reducing the ' woodiness ' and toughness of AFD-treated rabbit *longissimus dorsi* muscle would obtain when applied to a large commercial species of animal. The taste panel and tenderometer results amply confirmed that there were such benefits. Since ' woodiness ' was noted in non-dehydrated samples of beef from the control steers (i.e., of normal, low ultimate pH), however, it is possible that this adverse characteristic of AFD meat may be one of degree, rather than a specific effect of the process, and there are some preliminary indications that ' woodiness ' may become apparent even in meat of high ultimate pH on storage, although tenderness and colour may be retained at satisfactory levels.

An interesting aspect of the tenderometer results is the differential effect of high ultimate pH, and of the AFD process, between the different anatomical regions. Thus, the *semimembranosus*, which was toughest of the six muscles studied in the control steers, was of tenderness comparable to that of the *psoas* muscle in the treated steer. On the other hand, there was virtually no increase in tenderness in the *biceps femoris* muscle of the treated steer in comparison with that of the corresponding muscle of the control animal; yet the yield of fibrillar protein from this muscle, and its ease of breakdown on homogenisation, were relatively greater after treatment than with the case of *semimembranosus*. The AFD process caused a relatively greater increase in toughness (as measured in tenderometer) in the *semimembranosus*, deep pectoral and *longissimus dorsi* (thoracic) muscles of the treated steers; but in the control steer only the last-named muscle increased in toughness to a comparable degree. In view of the more marked responses of the taste panel to differences caused by the AFD process in both control and treated muscle, it must be assumed that the character measured by the tenderometer does not correspond exactly to the toughness or ' woodiness ' detected by the former. Another aspect of the differential response of the six muscle areas studied is exemplified by the reconstitution ratios. Although the various dehydrated muscles from the control steer absorb similar quantities of water on reconstitution, dehydrated *psoas major* and deep pectoral muscle from the treated steer appear to have improved their absorbing capacity to a relatively greater extent than did the other four muscles.

The results obtained for myofibrillar swelling with the various beef muscles also confirmed the view that the ultimate pH of the muscle is more important than the pH of the environment in which measurements are made. It has been found[12] that a protein fraction, which is soluble in 0·1M-potassium chloride when derived from a given muscle at an ultimate pH of 6·7, and hence sarcoplasmic in origin, was insoluble if the ultimate pH were 5·6, and was also denatured, since it

no longer dissolved when the pH was raised again to 6·7. The lowered water-holding capacity of the myofibrils derived from meat of low ultimate pH may be due to some intrinsic change in the properties of the myofibrillar protein and/or the precipitation of this sarcoplasmic protein on to the myofibrillar surface, as in pork muscle—when the rate of the *post mortem* glycolysis is very high.[13] Since the fraction concerned is denatured when the pH falls to 5·6, it is obvious why the subjection to a subsequent high environmental pH is of less significance with respect to water-binding than a high ultimate pH in the muscle itself.

The degree of response to high ultimate pH again differed between the muscles. The *longissimus dorsi* (lumbar) responded least and the *biceps femoris* responded most; in this respect they reflected their relative response to breakdown to the level of myofibrils, referred to above. It is noteworthy that, despite failure to detect an increased fibre diameter in the muscles of the treated steer before dehydration—indeed a marked decrease was found in *biceps femoris*—the fibre diameters *after* dehydration and reconstitution were consistently greater in the muscles of high ultimate pH (thus aligning with the data obtained with rabbit *longissimus dorsi*).

Since the glucose content of the meat with high ultimate pH was low, less deterioration in various attributes of eating quality during storage at 37° was expected. This was found with the treated meat which differed little from the corresponding samples stored at −20°. The control sample, on the other hand, markedly deteriorated. It is of interest, however, that browning had occurred in the treated samples, which suggests that even small quantities of glucose are sufficient to initiate the Maillard reaction.

The fact that the fibres of the treated and the control muscles could not be broken down easily after storage indicated that changes had occurred during this period leading to a denaturation of the protein in both. Yet, whatever the change, it had not appreciably affected the texture of the meat with a high ultimate pH.

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